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Supplemental Information

Inactivation of Both *foxo* and *reaper* Promotes Long-Term Adult Neurogenesis in *Drosophila*

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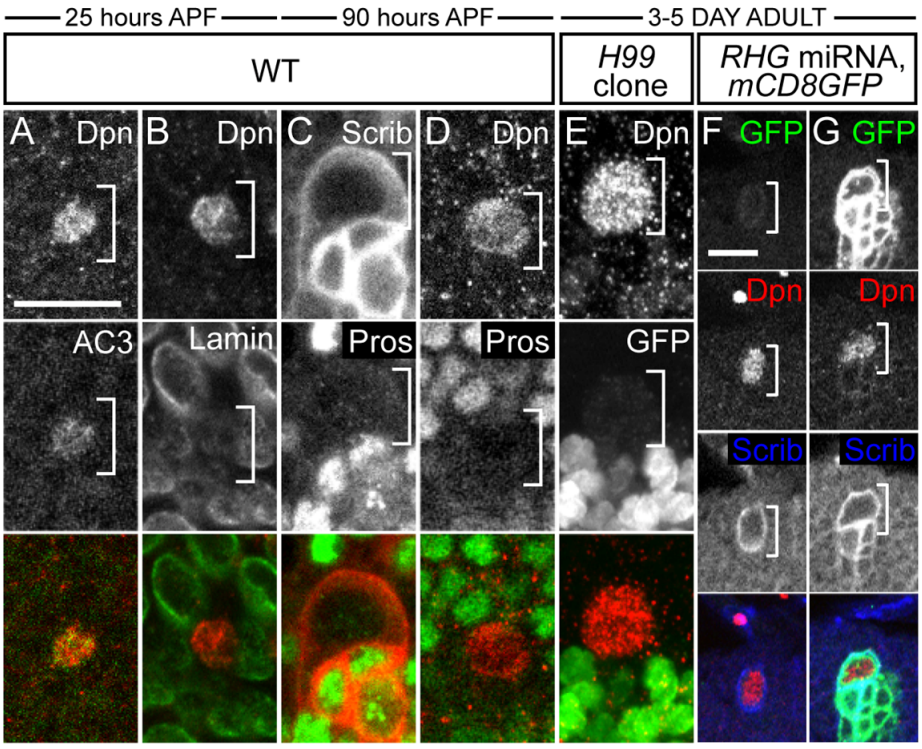

Figure S1. Some Neuroblasts Are Eliminated by Cell Death and Not by Pros-Dependent Terminal Differentiation

(A,B) Some CB neuroblasts other than mb neuroblasts also undergo cell death. Top two rows, single channel images with pseudo-colored overlay in bottom row, showing co-localization of markers in neuroblasts. AC3, activated caspase 3.

(C,D) Wild type (WT) mb neuroblasts after their terminal cell division fail to localize Pros to the nucleus. Mb neuroblasts before (C) and during (D) nuclear envelope breakdown.

(E) A single Dpn-expressing cell persists in *H99* mutant mb neuroblast clones. All GFP-expressing cells are *H99* homozygous mutant, including the mb neuroblast which only weakly expresses GFP.

(F,G) *worniu* transcription is attenuated in young *RHG* miRNA mb neuroblasts. Two *RHG* miRNA mb neuroblasts from the same brain lobe. Only one of the two mb neuroblasts expresses high levels of the mCD8:GFP reporter. Top three panels are single channel image and bottom row is overlay. (G). Neuroblasts in brackets. *worniuGAL4* is used to induce UAS transgene expression (F,G).

Scale bar (A,F) equals 10μm.

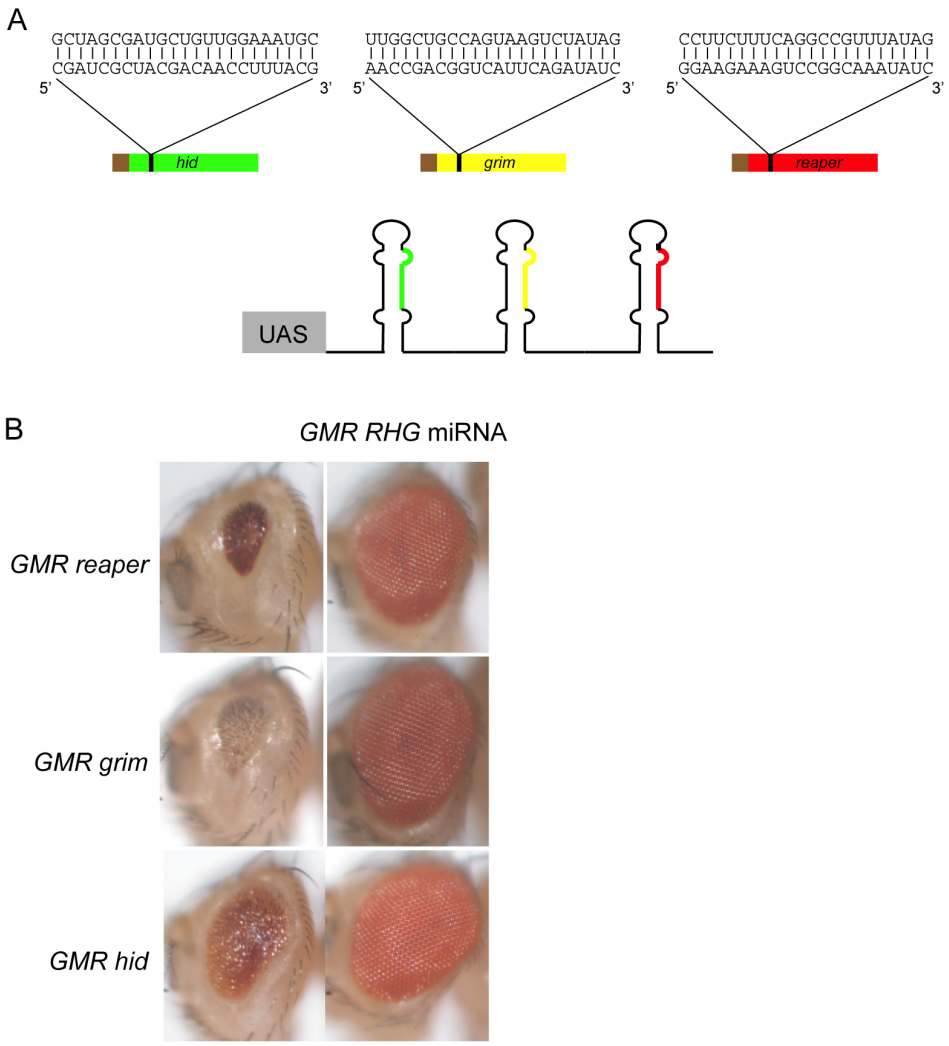

Figure S2. *RHG* miRNA Targets *rpr*, *hid*, and *grim* for Inactivation.
(A) Generation of *UAS-RHG* miRNA using the mir6.1 technique.
(B) Validation of *RHG* miRNA transgene. Overexpression of *RHG* miRNA can suppress *reaper*, *hid*, and *grim* induced cell death in the eye. GMR, glass multimer reporter.

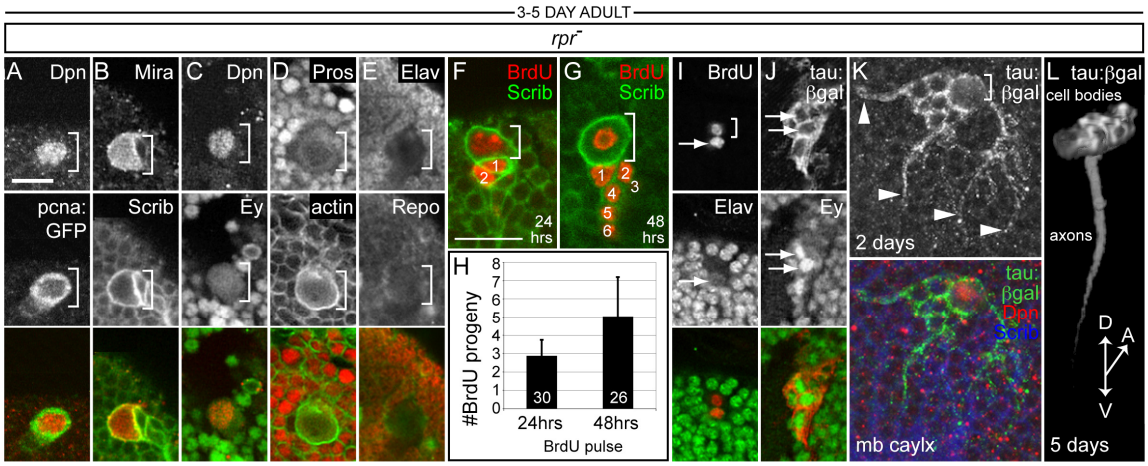

Figure S3. Mb Neuroblasts Persist in Young *rpr* Mutant Adults and Generate a Few New Adult mb Neurons

(A-G) Young (3-5 days old) *rpr* mutant adult mb neuroblasts (brackets) and their progeny (arrows, I-L).

(A-E, I-J) Top two rows, single channel images with colored overlay in bottom row, showing co-localization of markers in mb neuroblasts (A-E) and progeny (I,J).

(F-H) Mb neuroblasts incorporate BrdU and generate new progeny during a 24 (F) or 48 hour (G) BrdU pulse.

(H) Quantitation of the number of BrdU positive progeny generated during a BrdU pulse for indicated times. Number of BrdU positive mb neuroblasts scored, white numbers in black columns. Mean and std. dev.

(I,J) New adult progeny are Elav, Ey positive mb neurons, identified as BrdU positive progeny (arrow) or in clones (arrows, J) following activation of a *tau:LacZ* transgene in adult mb neuroblasts.

(K) Immature neurites (arrowheads) form from new adult mb neurons two days after *tau:LacZ* activation.

(L) A 50μm axon bundle extends through the mb peduncle at five days after *tau:LacZ* activation.

(A-E,I-L) and (F,G) same scale.

Scale bar (A,F) is 10μm.

Genotypes: (A) *pcna:eGFP;H99/XR38*, (B-I) *H99/XR38*, (J,L) *hsflp;act5c>stop>tau:LacZ;H99/XR38*, (K) *pcnaGAL4,UAS:flp/tubGAL80[ts], act5c>stop>tauLacZ;H99/XR38*.

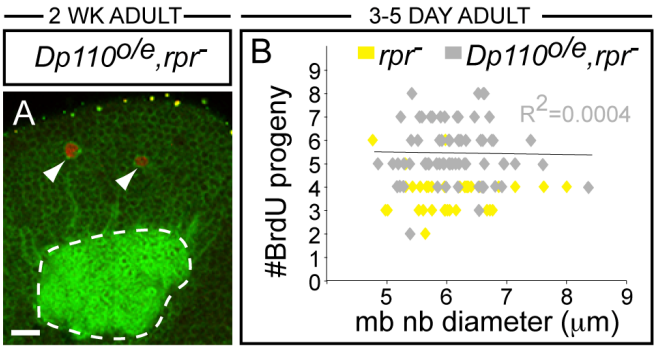

Figure S4. Reduced Levels of Insulin/PI3 Kinase Signaling Restricts *rpr* Mutant Adult mb Neuroblast Survival and Proliferation

(A) Z-projection of the dorsal brain surface showing persisting adult mb neuroblasts (arrowheads) near the mb calyx (white outline) following insulin/PI3 kinase pathway activation. Scale bar equals 10μm.

(B) Number of BrdU progeny generated in 24 hours relative to mb neuroblast cell size. Mb neuroblast cell size and proliferation rate do not correlate in young *Dp110$^{o/e}$,rpr^{-}* adults. R^2=coefficient of determination for a linear correlation. *worniuGAL4* induces *Dp110* overexpression (o/e) in mb neuroblasts.